

# **"Design, Synthesis, and Biological Evaluation of Pyrazolopyrimidine Derivatives"**

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**List of abbreviations:**

AIF	Apoptosis inducing factor
Apaf-1	Apoptotic protease activating factor 1
ATP	Adenosine triphosphate
BAK	Bcl-2 homologous antagonist/killer
BAX	Bcl-2-associated-x-protein
BR	Binding region
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthy
CAK	CDK-activating kinase
CDKs	Cyclin-dependent kinases
CVMAOS	Closed-vessel microwave assisted organic synthesis
DMSO-d <sub>6</sub>	Deuterated dimethyl sulphoxide
DTP	Developmental Therapeutic Program
E <sub>i</sub>	Electron impact
ELISA	Enzyme Linked Immunosorbent Assay
EtOAc	Ethyl acetate
FBS	Fetal bovine serum
GI <sub>50</sub>	Concentration of the compound causing 50% decrease in net cell growth
Het-C	Heteroatom-Carbon
IAP	Inhibitors of apoptosis
IC <sub>50</sub>	Half-maximal inhibitory concentration
kDa	Kilodalton
LC <sub>50</sub>	Lethal concentration (Concentration of the compound causing net 50% loss of initial cells)
MAOS	Microwave assisted organic synthesis

## ***List of abbreviation***

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MCR	Multicomponent reaction
MTP	Mitochondrial transmembrane potential
MWO	Microwave Oven
NCI	National Cancer Institute
NF- $\kappa$ B	Nuclear Factor kappa Beta
OD	Optical density
OVMAOS	Open-vessel microwave assisted synthesis
PBR	Phosphate-binding region
PBS	Phosphate Buffered Saline
PE	Petroleum ether
PKs	Protein kinases
PT	Permeability transition
PUMA	P53 upregulated modulator of apoptosis
RPMI	Roswell Park Memorial Institute medium
SD	Standard deviation
$SN_{2Ar}$	Nucleophilic substitution
SRB	Sulfo-Rhodamine-B
TCA	Trichloroacetic acid
TGI	Total growth inhibition
TNF	Tumor necrosis factor
TRAIL	Tumor necrosis factor-related apoptosis inducing ligand
Tz	Time zero

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**Abstract:**

**Title of thesis:**

**“Design, Synthesis, and Biological Evaluation of Pyrazolopyrimidine  
Derivatives”**

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# Abstract

By adopting fragmentic and structural hybrid approaches, a model of structural hybrid between pyrazolopyrimidine heterocyclic cores and *p*-substituted diaryl Schiff base fragment was designed. The design of small molecule was based on biologically multitarget cytotoxic lead structures like roscovitine, resveratrol to decrease drug resistance of cancer cells. The model represented by synthetic molecules **VIIa-t** and **IXa-i** were prepared by direct arylation of 4-chloropyrimidine derivatives **Va-f** with *p*-substituted phenolic aldehyde or *m*-substituted phenolic aldehyde either by direct nucleophilic substitution or under metal catalyzed assisted synthesis then condensation of the aldehyde products **VIa-f** and **VIIIa-f** with different aromatic amines, another model **Xa-t** was designed through alkylation of NH group of pyrazolopyrimidinone **IVa-f**. All synthetic steps were performed under open vessel microwave assisted synthesis conditions. Five compounds (**VIIIf**, **VIIIm**, **VIIo**, **Xj** and **Xr**) were selected by National Cancer Institute "NCI" ([www.dtp.nci.nih.gov](http://www.dtp.nci.nih.gov)); under the Developmental Therapeutic Program (DTP) USA for single dose screening program at 10  $\mu$ M in the full 60 cell panel. One compound (**VIIo**) was further screened at five different concentrations. All the target synthesized molecules (**VIIa-t** and **IXa-i**) were evaluated *in vitro* for their anti-proliferative activity against colon cancer cell line HCT-116 while synthetic molecules **Xa-t** screened against breast cancer MCF-7 at National cancer institute Egypt. Most of the synthesized compounds **VIIa-t** and **IXa-i** showed excellent antiproliferative activity ranging from 7.97  $\mu$ M to 74.43  $\mu$ M against HCT-116 cell line, while N-alkylated pyrazolopyrimidinone (**Xa-t**) derivatives showed a range from 8.22 and 34.78  $\mu$ M against MCF-7. In order to explore the mechanism of cytotoxicity, DNA-flow cytometry analysis for the effect of the molecules (**VIIo**, **IXi**) on HCT-116 cancer cell, which showed that these molecules arrested the cancer cells in G0/G1 phase, in addition, measurement of apoptotic regulator PUMA, BAX, caspase-3 level in colon cancer cell line after treatment with the molecule (**VIIo**, **IXi**) showed that they have a pro-apoptotic activity compared with control. Also two compounds (**Xj**, **Xr**) from the second series were analyzed by cell cycle flow analysis where they showed cell cycle arrest of breast cancer MCF-7 mainly in G0/G1 phase. Furthermore, CDK2 kinases enzyme inhibition % assay was done for molecules (**VIIe**, **VIIo**, **VIIIt**, **IXd**, **IXe**, **IXh**, **IXi**) and IC<sub>50</sub> for the molecules (**IXd**, **IXe**, **IXh**, **IXi**) showed selective inhibition CDK2. Finally, to investigate cell cycle arrest of HCT-116 due to effect of synthetic molecule, western blot analysis for expression of CDK-2, cyclin A, P53, P21 and P27 was performed in dose dependent manner that suggested this molecule (**VIIo**) may be CDK

pathway inhibitor one of its targets. As conclusion, in this investigation, a different series of pyrazolopyrimidine derivatives were discovered as potential cytotoxic agents against HCT-16 and MCF-7 cancer cells.

The structures of final compounds were confirmed by various spectral and microanalytical data.

**The study involved the synthesis of the following reported unavailable intermediates:**

1] 4-Methoxyphenyl hydrazine (Ib)

2] 4-Chlorophenyl hydrazine (Ia)

3] Ethoxymethylidene malononitriles (IIa)

4] Ethoxyethylidene malononitriles (IIb)

**The study involved the synthesis of the following reported unavailable intermediates under OVAMS as noval procedure to literature:**

1] 5-Amino-1-phenyl-1H-pyrazole-4-carbonitrile (IIIa)

2] 5-Amino-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile (IIIb)

3] 5-Amino-1-(4-chlorophenyl)-1H-pyrazole-4-carbonitrile (IIIc)

4] 5-Amino-1-(4-chlorophenyl)-3-methyl-1H-pyrazole-4-carbonitrile (IIId)

5] 5-Amino-1-(4-methoxyphenyl)-1H-pyrazole-4-carbonitrile (IIIe)

6] 5-Amino-1-(4-methoxyphenyl)-3-methyl-1H-pyrazole-4-carbonitrile (IIIf)

7] 1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVa)

8] 3-Methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVb)

9] 1-(4-Chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVc)

12] 1-(4-Chlorophenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVd)

13] 4-Chloro-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (Va)

14] 4-Chloro-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (Vb)

15] 4-Chloro-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidine (Vc)

16] 4-Chloro-1-(4-chlorophenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidine (Vd)

**In addition, the study involved the synthesis of the following novel intermediates.**

1] 4-Chloro-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidine (Ve)

2] 4-Chloro-1-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d] pyrimidine (Vf)

3] 1-(4-Methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVe)

4] 1-(4-Methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVf)

5] 4-(4-Methanoylphenoxy)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (VIa)

6] 4-(4-Methanoylphenoxy)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (VIb)

7] 1-(4-Chlorophenyl)-4-(4-methanoylphenoxy)-1H-pyrazolo[3,4-d]pyrimidine (VIc)

8] 1-(4-Chlorophenyl)-4-(4-methanoylphenoxy)-3-methyl-1H-pyrazolo[3,4-d]pyrimidine (VIId)

9] 4-(4-Methanoylphenoxy)-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d] pyrimidine (VIe)

10] 4-(4-Methanoylphenoxy)-1-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d] pyrimidine (VI f)

11] 1-(4-Chlorophenyl)-4-(3-methanoylphenoxy)-1H-pyrazolo[3,4-d] pyrimidines (VIIIa)

12] 1-(4-Chlorophenyl)-4-(3-methanoylphenoxy)-3-methyl-1H-pyrazolo[3,4d]pyrimidines (VIIIb)

13] 4-(3-Methanoylphenoxy)-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidines (VIIIc)

14] 4-(3-Methanoylphenoxy)-1-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d] pyrimidine (VIIId)

**Also, the study involved the synthesis and characterization of the following new target compounds:**

1] 1-(4-Chlorophenyl)-4-[4-(phenyliminomethyl)phenoxy]-1H-pyrazolo[3,4-d]pyrimidine (VIIa)

2] 4-[4-(4-Bromophenyliminomethyl)phenoxy]-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidine (VIIb)